

PAPER CHROMATOGRAPHIC STUDIES IN THE ALOINEAE

I. PRELIMINARY OBSERVATIONS ON SOME SPECIES OF HAWORTHIA

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ABSTRACT

One-dimensional paper chromatograms were prepared from several species and varieties of the Coarctatae Section of *Haworthia*, and fluorescent spots were studied. Identical patterns were obtained from *H. greenii* forma *bakerii* and two clones of *H. greenii* forma *pseudocoarctata*. The pattern of *H. fulva* was close to but not identical with those of the two varieties of *H. greenii* and was the same as that of *H. coarctatoides* except that it contained one more spot. *H. baccata* had a pattern that differed greatly from the others. Identical patterns were found for *H. reinwardtii* var. *committeensis*, var. *tenuis*, and possibly for var. *diminuta*. *H. reinwardtii* vars. *chawinii* and *haworthii* were very similar, but vars. *archibaldiae* and *valida* differed from one another and from the other varieties. The possible importance of this method to taxonomy is discussed.

INTRODUCTION

Classical methods of plant taxonomy, utilising morphological characters and especially the structure of the flowers, have been employed by botanists for several centuries and have always been accepted as the basic methods of identifying and classifying plants. In more recent years, several other methods have been developed that provide other types of information that do not supplant but that can frequently supplement the classical ones. Studies of chromosome structure and number and the biosystematic correlation of the techniques of cytology, ecology, and plant geography have been useful adjuncts to morphological methods and have upon some occasions furnished valuable information on several difficult taxonomic problems. Recently, relationships among various plants and animals have been studied by a comparison of some of their chemical compounds as shown by paper chromatography. This method has now been applied to a fairly large number of different organisms and the early results have been encouraging. Whether it will ultimately prove of con-

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siderable importance can not be stated clearly as yet, but preliminary reports indicate that a more extensive trial upon a larger number of plants and animals is justified.

REVIEW OF THE LITERATURE

Some of the early work on paper chromatography as applied to taxonomy was carried out on *Drosophila melanogaster*. Hadorn and Mitchell (1951) mashed whole boiled pupae or adults on filter paper, used the one-dimensional descending method and a solvent consisting of two parts of n-propanol and one part of 1% ammonia, and analysed the tracks for fluorescent and ninhydrin-positive substances. Buzzati-Traverso (1953a, 1953b) showed that strains of *Drosophila melanogaster* of different genotypes give constant and distinctive patterns and that by chromatography heterozygotes can readily be distinguished from either parental homozygous strain even though no phenotypic difference can be observed between the heterozygote and the homozygous dominant. He cautioned that R_f values are not highly accurate because the spots are large and the location of the solvent front is, necessarily, somewhat arbitrary.

In *Drosophila melanogaster* Fox (1956) was able to identify the spots chemically and to show that the presence of a peptide differentiated males from females. He pointed out that the one-dimensional method should be followed by the two-dimensional method, as the latter may reveal spots that do not appear with the former.

Buzzati-Traverso and Rechnitzer (1958) studied the fluorescent and ninhydrin-positive patterns from muscles, liver, and the lens of the eye of different species of fish. The patterns derived from the muscles showed differences that were constant and easily recognizable and the patterns were remarkably constant irrespective of the size and age of the fish. An important relationship was shown by the various species, for the closer the taxonomic position of any two or more species, the greater was the similarity of their chromatographic patterns. The authors stated, "This technique will become a useful tool in taxonomic and population-genetical studies."

Because of differences in paper chromatography, the syringaldehyde/vanillin values, the Maule tests, the HCl/methanol reaction, and manganese and iron content, Towers and Gibbs (1953) suggested that the *negundo* group of the genus *Acer* should be separated from the other species as the genus *Negundo*.

Selle (1954) applied paper chromatography to a study of the "quick decline" virus infection of orange trees. The sweet orange is grafted on the rootstock of some other variety of citrus fruit and its tolerance to the virus varies with the rootstock. The disease is usually fatal only if the rootstock is from the sour

orange, *Citrus aurantium*. Chromatograms were made from the bark of various types of rootstocks budded with valencias or navel oranges. If the rootstock was from the sour orange, a yellow, fluorescent, flame-like spot with an R_f value of approximately 0.50 was present, but if it was from another species of citrus trees, this spot was missing. By this method it is possible to determine which trees in an orchard are growing on a sour orange rootstock and therefore are susceptible to the infection.

Micks (1954) used paper chromatography to study mosquitoes, as many taxonomic problems in the group are not susceptible of analysis by the usual methods. Different chromatographic patterns of the free amino acids were obtained for different genera, and in the *pipiens* complex the patterns of the various species were similar but the intensities of the spots were strikingly different. This difference in intensity indicates, apparently, that there are differences in the concentrations of the amino acids. Micks states, "it appears from the results thus far obtained that paper chromatography may be an extremely useful taxonomic tool, particularly when there are no good morphological recognition characters for the species involved."

Kirk et al. (1954) studied chromatographic patterns from the posterior or lateral edges of the foot of *Theba pisana*, *Helix aspersa*, *Bothriembryon indutus*, *B. kingii*, *B. dux*, *B. leeuwinensis*, and *Austrosuccinea contenta*, all land snails from Australia. Each species was easily distinguished by its ultra-violet absorption and fluorescence patterns. Specimens of the same species from geographical localities as much as 300 miles apart showed no differences and the patterns are uninfluenced by a wide range of environmental conditions or by age. Kirk et al. suggested that with further refinement and greater ability to identify and measure quantitatively some of the spots in the patterns, it might be possible to evaluate degrees of relationship between closely related species.

In 1957, Robertson used paper chromatography to study taxonomy in the Coleoptera, Lepidoptera, Diptera, and Hymenoptera. Apparently interspecific differences can be detected but there did not appear to be definite enough criteria to distinguish the various orders of insects.

In mangoes, the fruit is ordinarily important in classification, but it would be highly desirable if vegetative parts could be used, since many mangoes bear fruit only in alternate years and it is sometimes desirable to identify young trees before they reach the bearing age. Teas et al. (1959) studied twenty-one varieties; seven gave chromatographic patterns that were entirely different from every other one and the other fourteen fell into four groups of from two to five varieties each. Teas and his co-workers feel that this method holds some promise in a programme for the identification of varietal differences. Some of the individuals that give the same patterns with the particular technique used might show different patterns with other sprays and other solvents.

In the American genus *Baptisia*, Turner and Alston (1959a, 1959b) demonstrated that distinctive profiles could be obtained from *B. laevicaulis* and *B. viridis*. Hydrolysed and unhydrolysed extracts of petals were applied to the paper by streaking and were developed by descending and ascending methods. Several solvents were used and it was shown that, in a hybrid swarm, recombination of specific biochemical compounds peculiar to each parental type occurs. Birdsong, Alston, and Turner (1960) used paper chromatography to demonstrate the amino acid canavanine in seeds of members of the Leguminosae, the only family in which it occurs, and Tschiersch (1959) reported the synthesis of this compound in the South African Leguminous genus *Canavalia*.

Some work has been carried out recently on South African members of the Iridaceae (Riley and Bryant, 1959, 1961). Twenty different major fluorescent spots were distinguished. The fundamental pattern was the same for six species of *Watsonia* but no two species had exactly the same pattern; the tracks of the six species of *Watsonia* show much greater similarity to one another than to those of *Dietes grandiflora*, *Babiana bainesii*, and *Sparaxis aureum*.

Studies in the Dipterocarpaceae by Bate-Smith and Whitmore (1959) covered twenty-eight species from several genera and especially from *Shorea*, the largest genus of the family. The presence or absence of certain phenolic constituents was used as a basis for specific differentiation, and the differences were enough to be the basis for the identification of each species.

Paper chromatography was recently applied to a study of species and subspecies of *Populus* (Börtitz, 1962). The expressed sap of the leaves was treated with several solvent systems and the fluorescent patterns were observed without identification of the separated compounds. The various species and subspecies differ more or less in quantity, position, colour, and intensity of the fluorescent spots or "zones". The closer the relationships between the taxa that were studied, the more alike were the chromatographic patterns. The age of the tree is of no importance and leaves from the top and from the middle of a twig gave the same pattern. Börtitz considers that his results of "chemical taxonomy" are similar to the natural system.

In the present paper, observations on some species and varieties of the Coarctatae Section of the genus *Haworthia* are presented. In April, 1961, they were read at a meeting of the Association of Southwestern Biologists (Isbell and Riley, 1961), and in September, 1962, a report on some species of the Rigidiae and Retusae Sections was presented to the International Conference on Taxonomic Biochemistry, Physiology, and Serology (Riley and Hopkins, 1962).

The taxa studied along with the writer's voucher numbers and the numbers of the International Succulent Institute from which the plants were received are:

<i>H. baccata</i> —58132—ISI-B0301	<i>H. reinwardtii</i> var.
<i>H. coarctatoides</i> —58142—ISI-C2301	<i>archibaldiae</i> —5879—ISI-R0805
<i>H. fulva</i> —58130—ISI-F2901	<i>valida</i> —5878—ISI-2601
<i>H. greenii</i> forma	<i>committeensis</i> —58119—ISI-R1201
<i>bakerii</i> —58139—ISI-G3202	<i>tenuis</i> —58115—ISI-R4801
<i>H. greenii</i> forma <i>pseudocoarctata</i>	<i>diminuta</i> —5875—ISI-R5001
clone 6—58136—ISI-G3406	aff. <i>chalwinii</i> —58122—ISI-R1124
clone 12—58135—ISI-G3412	<i>haworthii</i> —58126—ISI-R1611

MATERIAL AND METHOD

Seven varieties of *Haworthia reinwardtii* and four other species of the Coarctatae Section were studied. They had been received from the International Succulent Institute at Millbrae, California, through the courtesy of Mr. J. W. Dodson, who obtained them from South African botanists. They were grown in pots in a mixture of fine white sand (capable of passing through a 30-mesh screen) and peat moss to which had been added commercial fertilizer according to the practice recommended by the University of California Agricultural Experiment Station Extension Service (Baker 1957). The use of a uniform method of culture reduced greatly the possibility that some spots of some species or varieties might be the result of the conditions under which the plants were grown.

Sheets of Whatman No. 1 chromatographic paper about 55 cm. long were cut lengthwise into two pieces about 235 mm. wide. A line was drawn about 75 mm. from the top and four smears of leaf tissue were made along this line at intervals of 5 cm. Each smear included both mesophyll and epidermis, was pressed into the paper by the rounded end of a test tube, and formed a spot approximately 10–15 mm. in diameter. The spots were allowed to dry at least 24 hours but could be kept for two or three days without apparently undergoing any change.

The solvent system was prepared by putting into a separatory funnel 160 ml. n-butanol, 200 ml. distilled water, and 40 ml. glacial acetic acid in that order; the mixture was shaken 2 min. and set aside for 1 hr. to separate. The lower phase of the mixture was then placed in a tray at the bottom of the chamber of a chromatographic cabinet ("chromatocab"); the chromatograms were hung in the solvent trays, the chromatocab was sealed, and the chromatograms were allowed to equilibrate with the lower phase for at least one hour. After the time had expired, the upper phase of the solvent mixture was added to the solvent trays and the solvent front was allowed to descend until it was about 25 mm. from the lower edge of the paper. This process normally required 22–24 hours. When the solvent front had descended to the required level, the chromatocab

was opened and the chromatograms were removed and dried 24 hours in the air. The above steps were carried out in a room that maintained a constant temperature of $27^{\circ} \pm 1^{\circ}\text{C}$.

After the chromatograms had dried in the air, they were placed in an oven at 101°C and kept there 72 hours; this step is important as it intensifies the fluorescent tracks. After three or four days in the oven, the chromatograms were placed under a twin-tube Black-ray BLB-15 ultraviolet lamp with main emission in the $3660 \text{ \AA}$ range. The spots were outlined in pencil whilst the chromatogram was under the ultraviolet lamp and the solvent front was marked; the colours of the spots were recorded on the chromatograms at this time. The R_f values were then computed for each spot of each track and represent the linear distance from the centre of the spot to the original line divided by the distance from the original line to the solvent front. To determine the consistency of the results, two sheets of four tracks each were prepared for each plant. Each of the eight tracks was made from a different leaf.

OBSERVATIONS

When the chromatograms were observed under ultra-violet light, fluorescent spots immediately appeared which varied in number from five to ten. Most of them were blue, but some were pink. It soon became obvious that in each species or variety the spots formed a definite chromatographic pattern or "biochemical profile" that was characteristic of the taxon. Three problems then arose. Do leaves from all parts of the plant produce the same pattern? Are the patterns of different plants of the same species or variety the same? Does a given plant produce identical patterns at different times? If any of these questions is answered in the negative, the method is utterly worthless for a taxonomic study.

To answer the first question, eight leaves were tested on each plant. Four leaves were smeared on a chromatographic sheet and two sheets were run for each plant. The patterns of all eight leaves of any given plant were identical and the R_f values varied but little. The eight patterns were so uniform that it seems unnecessary to illustrate them in all the taxa, but figure 1 shows the patterns of all eight leaves of *H. greenii* forma *bakerii* and of *H. reinwardtii* var. aff. *chalwinii* by way of illustration. As stated previously, the piece of tissue that was smeared always included part of the mesophyll and the leaf epidermis. The latter was too thin to use alone and when the former was used alone, few fluorescent spots appeared.

The second question was difficult to answer because usually only one plant of a species or variety was available. Fortunately, two different clones of *H. greenii* forma *pseudocoarctata* could be tested and they produced identical profiles (Fig. 2) thus supporting an affirmative answer to the question.

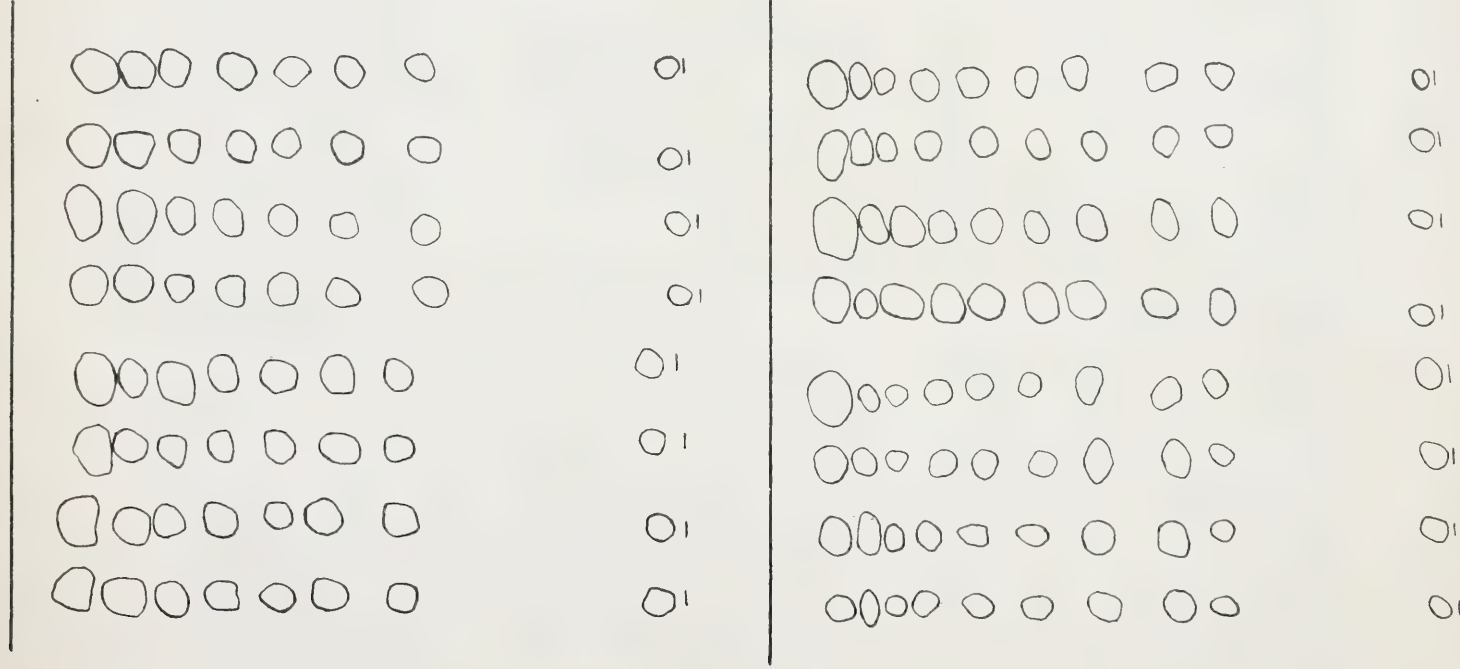


FIG. 1.—Chromatographic patterns of eight leaves of *Haworthia greenii* forma *bakerii* (above) and of *H. reinwardii* var. *aff. chalcidii* (below). (Slightly reduced).

Three plants were used to test the third question. Chromatograms were run for *H. fulva*, *H. reinwardtii* var. *archibaldiae*, and *H. reinwardtii* var. *haworthii*. Each was tested in the Autumn of 1959 and again approximately six months later in May, 1960 (Fig. 3). Since the two series of patterns were indistinguishable for each species, it seems obvious that they are constant features of a plant, at least over a period of six months. This point is important because, in general, fluorescent compounds are less stable than some other kinds that could be studied and some investigators on other genera have found them to be too

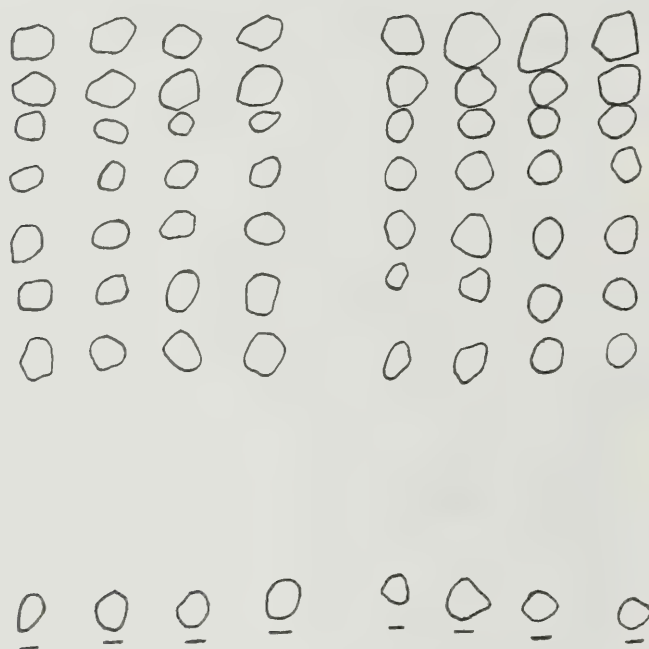


FIG. 2.—Chromatographic patterns of four leaves each of *Haworthia greenii* forma *pseudocoarctata*, Clone No. 6 (left) and Clone N. 12 (right). (Slightly reduced).

evanescent to be reliable for a critical study. The absence of differences in *Haworthia* over a six-months period may result from the fact that it is a very slow-growing genus and that therefore these compounds are much more stable than they are in more rapidly-growing plants of a non-succulent nature.

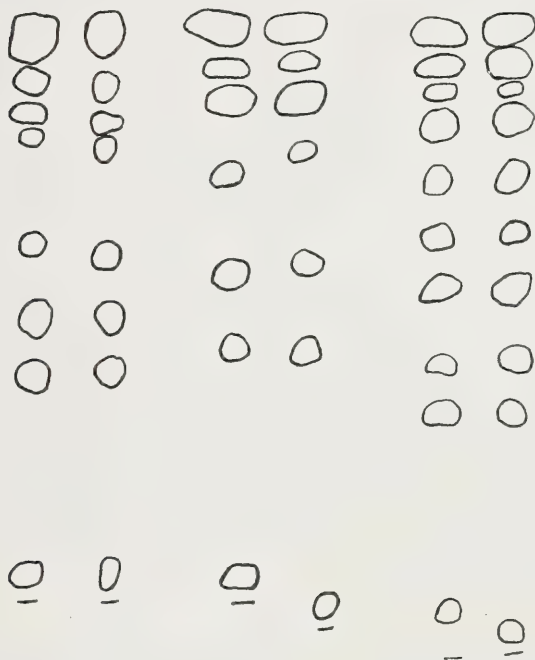


FIG. 3.—Chromatographic patterns of typical leaves of *Haworthia fulva* (left), *H. reinwardtii* var. *archibaldiae* (centre) and *H. reinwardtii* var. *haworthii* (right); the left-hand track of each pair was made in the Autumn of 1959 and the right-hand track in the Spring of 1960. (Slightly reduced).

To facilitate a comparison of the various species and varieties, an attempt was made to distinguish each spot on the basis of R_f value and colour; thus, twenty-seven spots have been recognised and are listed in Table 1. All are invisible in ordinary light and the colours that have been observed under the ultra-violet lamp are light blue, medium blue, dark blue, bright blue, and pink; the R_f values range from 0.075 to 0.682. In addition to these spots, one other is present that is green in ordinary light and red in ultra-violet light; it moves rapidly down the paper, is just at or behind the solvent front, and has an R_f value of around 0.95. This is a spot of chlorophyll and, since it is present in the same position in each taxon, has been omitted from any consideration. The other twenty-seven spots are somewhat arbitrary since it has not yet been

TABLE 1.

The twenty-seven spots recognised in the *Haworthia* species studied with their R_f values and colours.

No.	R _f Value	Colour	No.	R _f Value	Colour	No.	R _f Value	Colour
1	·075—·124	BB	10	·234—·241	MB	19	·467—·493	P
2	·082—·102	LB	11	·234—·275	P	20	·477—·511	BB
3	·112—·123	MB	12	·264—·321	LB	21	·513—·533	BB
4	·129—·186	DB	13	·269—·329	MB	22	·543—·567	LB
5	·148—·164	P	14	·340—·356	MB	23	·550—·596	MB
6	·166—·215	P	15	·348—·393	P	24	·564—·597	BB
7	·201—·216	LB	16	·378—·410	MB	25	·577—·614	MB
8	·207—·248	P	17	·411—·455	LB	26	·606—·629	BB
9	·223—·272	BB	18	·413—·492	MB	27	·611—·682	MB

BB = bright blue; LB = light blue; MB = medium blue; DB = dark blue; P = pink.

possible to determine their chemical nature and since there is some overlapping of R_f values. That R_f values are considerably variable has been pointed out by Buzzati-Traverso (1953b), and it is possible that in one or two instances two or three spots are really one, and that perhaps some spots are actually two or three. Thus, spots 5, 6, 8, and 11 may all represent one compound since they are all pink, since the ranges of 5 and 6 almost touch, and since there is some overlapping between 6 and 8 and between 8 and 11, but if they are one compound they cover a very much larger range of R_f values (0·148 to 0·275) than do most spots; until a chemical analysis can be made, they will be regarded as four spots. When an identification of the spots can be made chemically, many problems such as this may be resolved.

TABLE 2

The spots (numbered according to Table 1) found in various species, forms and varieties of *Haworthia* with their mean R_f values; each mean R_f value is the average of the R_f values from eight tracks.

<i>baccata</i>	<i>coarctatoides</i>	<i>fulva</i>	<i>greenii</i> forma	<i>greenii</i> forma	<i>pseudocoarctata</i>
			<i>bakerii</i>	clone 6	clone 12
2—·092	1—·099	1—·111	1—·112	1—·112	1—·112
7—·209	6—·181	6—·187	6—·180	6—·199	6—·180
17—·436	9—·250	9—·242	11—·245	11—·238	11—·239
22—·557	18—·436	12—·291	13—·318	13—·312	13—·314
	23—·565	18—·452	16—·399	16—·401	16—·396
	27—·641	23—·574	19—·483	19—·481	19—·482
		27—·661	25—·592	25—·580	25—·600
<i>H. reinwardtii</i> var.					
<i>archibaldiae</i>	<i>valida</i>	<i>commiteensis</i>	<i>tenuis</i>	<i>diminuta</i>	<i>chalconii</i>
1—·093	1—·106	1—·096	1—·103	1—·090	1—·092
4—·175	6—·181	4—·143	4—·157	4—·140	4—·139
8—·235	10—·237	8—·224	8—·223	6—·203	6—·177
13—·327	13—·314	11—·267	11—·264	11—·255	8—·231
18—·481	16—·391	14—·346	14—·348	13—·311	13—·303
25—·593	20—·487	18—·416	18—·427	16—·397	15—·389
	25—·587	21—·517	21—·526	20—·493	18—·469
		26—·610	26—·624	24—·580	23—·578
					27—·655
					27—·654

On the basis of these twenty-seven different spots, certain similarities between different taxa are readily discernible (Table 2 and Fig. 4). *Haworthia baccata* has only four spots (in addition to chlorophyll) and differs radically from all the other species and varieties. In addition, a light blue spot (no. 2) replaces the bright blue spot (no. 1) found near the original line in all the other taxa, but it is possible that this is a quantitative rather than a qualitative difference. Also, this species contains no pink spot, whereas all the other taxa that have been studied do (nos. 6, 8, 11). *H. coarctatoides* has six spots. Its pattern is almost identical with that of *H. fulva* but it lacks light blue spot no. 12. It is closer on this basis to *H. fulva* than it is to any other taxon. *H. fulva* and the forms of *H. greenii* have seven spots each, but, with the exception of the first two spots (nos. 1 and 6), they are all different. These differences are probably real ones with the possible exception of nos. 16 and 18 and nos. 25 and 27. At least, the differences are great enough to assume that *H. fulva* is closer to *H. coarctatoides* than it is to *H. greenii*. It has been pointed out that clones 6 and 12 of *H. greenii* forma *pseudocoarctata* have identical biochemical profiles and it is interesting to note that the patterns of *H. greenii* forma *bakerii*, and *H. greenii* forma *pseudocoarctata* are identical, which resemblance probably indicates that this method is better for differentiating species than varieties of the same species. In Figure 4, only typical tracks of each taxon have been portrayed to avoid too much redundancy.

Seven varieties of *H. reinwardtii* have been studied and the relationships on the basis of chromatographic patterns are interesting. *H. reinwardtii* var. *archibaldiae* is clearly distinct from the other varieties as it has only six spots. *H. reinwardtii* var. *valida*, with seven spots, is also different from the other varieties. Two varieties, *committeensis* and *tenuis*, have identical chromatographic patterns and on this basis appear to be closely related. They have eight spots, as does also *H. reinwardtii* var. *diminuta*. These three varieties have a number of spots in common and their differences may result from a misunderstanding of the nature of the spots. Thus, if spots 6 and 8 are really one, and if spots 13 and 14, spots 16 and 18, spots 20 and 21, and spots 24 and 26 are actually the same, these three varieties have identical patterns. The members of each of these pairs of spots are of the same colour but have R_f values that, with the exception of 6 and 8, do not overlap. It is quite possible that these chromatographic patterns indicate that varieties *committeensis*, *diminuta*, and *tenuis* are closely related. *H. reinwardtii* var. *chalwinii* and *H. reinwardtii* var. *haworthii* are also very similar to one another. Each has nine spots and seven of them are identical. Furthermore, it is possible that spots no. 5 and 6 are actually the same; if so, there is only one clear-cut difference between the two varieties and they must be closely related.

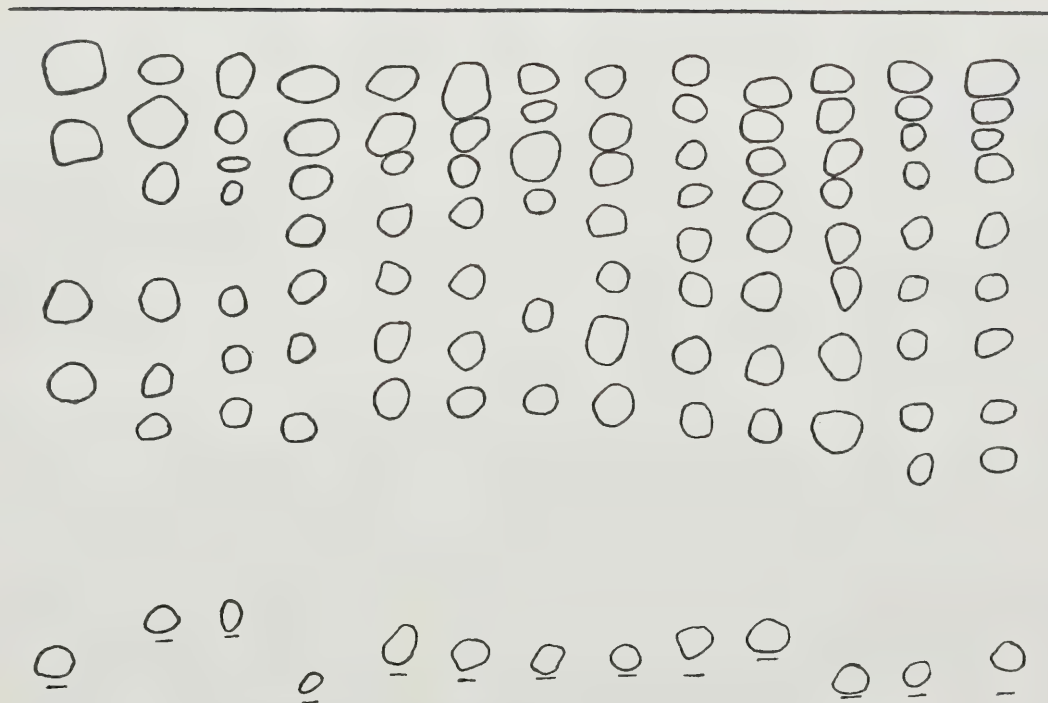


FIG. 4.—Chromatographic patterns of typical leaves of *Haworthia* taxa. From left to right, they are from: *H. baccata*; *H. coarctatoides*; *H. fulva*; *H. greenii* forma *bakerii*; *H. greenii* forma *pseudocoarctata* Clone 6; *H. greenii* forma *pseudocoarctata* Clone 12; and *H. reinwardtii* vars. *archibaldiae*, *valida*, *committeensis*, *tenuis*, *diminuta*, *chalwinii*, and *haworthii*. (Slightly reduced).

H. reinwardtii var. *diminuta*

·086	·086	·092	·085	·095	·097	·095	·090	BB
·135	·132	·131	·129	·148	·150	·149	·149	DB
·200	·200	·198	·196	·207	·209	·206	·208	P
·263	·263	·263	·263	·249	·251	·246	·249	P
·307	·307	·306	·306	·317	·316	·316	·319	MB
·379	·378	·398	·392	·410	·409	·408	·408	MB
·474	·477	·477	·481	·570	·511	·509	·511	BB
·564	·565	·567	·573	·592	·593	·592	·599	BB
·960	·958	·956	·956	·958	·955	·956	·956	R

H. reinwardtii var. aff. *chalinii*

·090	·090	·089	·085	·098	·098	·096	·096	BB
·134	·141	·134	·132	·143	·143	·143	·143	DB
·181	·181	·169	·169	·178	·178	·182	·182	P
·248	·248	·224	·224	·225	·225	·230	·230	P
·310	·310	·307	·297	·301	·301	·301	·301	MB
·387	·387	·390	·382	·391	·389	·393	·393	P
·458	·458	·465	·453	·481	·481	·481	·477	MB
·562	·562	·565	·561	·596	·596	·596	·594	MB
·654	·654	·650	·650	·660	·660	·660	·657	MB
·949	·951	·949	·949	·975	·975	·975	·975	R

H. reinwardtii var. *haworthii*

·082	·082	·082	·080	·074	·079	·075	·075	BB
·112	·123	·123	·112	·115	·117	·115	·115	MB
·155	·164	·164	·155	·151	·151	·148	·148	P
·215	·215	·211	·211	·211	·211	·206	·206	P
·301	·301	·301	·284	·277	·277	·269	·269	MB
·378	·378	·378	·378	·355	·355	·348	·348	P
·462	·462	·462	·450	·415	·428	·421	·419	MB
·562	·562	·562	·552	·570	·570	·558	·558	MB
·658	·658	·650	·650	·664	·662	·648	·648	MB
·935	·935	·930	·933	·966	·966	·944	·942	R

In Table 3 are listed the R_f values and colours of all eight spots of each of the species and varieties that were studied.

As in some of the earlier studies by various investigators, this method of paper chromatography seems to show some promise of usefulness in taxonomic work. It is still in early stages of development and will undoubtedly need to be improved. The use of other solvent systems and the two-dimensional method will furnish additional information but probably the biggest advance will come from the chemical identification of the spots.

REFERENCES

- BAKER, K. F., 1957. "The U.C. system for producing healthy container grown plants." *Calif. Agric. Exp. Sta. Extension Service Manual* 23.
- BATE-SMITH, E. C. and T. C. WHITMORE. (1959). "Chemistry and taxonomy in the Dipterocarpaceae." *Nature* 184, 795-796.
- BIRDSONG, B. A., R. ALSTON and B. L. TURNER. (1960). "Distribution of canavanine in the family Leguminosae as related to phyletic groupings." *Canad. Jour. Bot.* 38, 499-505.
- BÖRTITZ, SIEGFRIED. (1962). "Papierchromatographische Differenzierung einiger Arten und Sorten der Gattung *Populus*," *Der Züchter* 32, 24-33.
- BUZZATI-TRAVERSO, ADRIANO A. (1953a). "Paper chromatographic patterns on genetically different tissues: a contribution to the biochemical study of individuality." *Proc. National Acad. Sci. (Washington)* 39, 376-391.
- (1953b). "Identification of recessive gene heterozygotes by means of paper-chromatography," *Nature* 171, 575-576.
- and A. B. RECHNITZER. (1953). "Paper partition chromatography in taxonomic studies." *Science* 117, 58.

- FOX, ALLEN S. (1956). "Application of paper chromatography to taxonomic studies." *Science* **123**, 143.
- HADORN, ERNST and H. K. MITCHELL. (1951). "Properties of mutants of *Drosophila melanogaster* and changes during development as revealed by paper chromatography." *Proc. National Acad. Sci. (Washington)* **37**, 650-665.
- ISBELL, C. J. and H. P. RILEY. (1961). "Chromatographic studies in the Coarctatae Section of the genus *Haworthia*. A chemo-taxonomic study." Abstract. *Bull. Assoc. Southeastern Biologists* **8**, 23.
- KIRK, R. L., A. R. MAIN and F. G. BEYER. (1954). The use of paper partition chromatography for taxonomic studies of land snails. *Biochem. Jour.* **57**, 440-442.
- MICKS, DON W. (1954). Paper chromatography as a tool for mosquito taxonomy: the *Culex pipiens* complex. *Nature* **174**, 217-218.
- RILEY, H. P. and T. R. BRANT. (1959). Preliminary studies in the identification of species by the paper chromatography of fluorescent compounds. *Proc. 9th Internat. Bot. Cong.* **2**, 327.
- (1961). The separation of nine species of the Iridaceae by paper chromatography. *Amer. Jour. Botany* **48**, 133-137.
- and J. D. HOPKINS. (1962). Paper chromatographic studies in the Aloineae II. The Rigidiae and Retusae Sections of *Haworthia*. Abstract. *Internat. Conf. Tax. Biochem., Physiol. and Serology*, **51**.
- ROBERTSON, J. G. (1957). Paper chromatography in insect taxonomy. *Canad. Jour. Zool.* **35**, 411-419.
- SELLE, R. M. (1954). Determination of sour orange rootstock by paper chromatography. *Nature* **174**, 140-141.
- TEAS, H. J., W. ALMEYDA and H. F. WINTERS. (1959). Identification of mango varieties by paper chromatography. *Proc. Assoc. Southern (U.S.) Agric. Workers* 1959, 223.
- TOWERS, G. H. N. and R. D. GIBBS. (1935). Lignin chemistry and the taxonomy of higher plants. *Nature* **172**, 25-26.
- TSCHIRSCH, B. 1959. "Ueber canavanin." *Flora* **147**, 405-416.
- TURNER, B. L. and R. ALSTON. (1959a). Segregation and recombination of chemical constituents in a hybrid swarm of *Baptisia laevicaulis* x *B. viridis* and their taxonomic implications. *Amer. Jour. Botany* **46**, 678-686.
- (1959b). Applications of paper chromatography to systematics: Recombination of parental biochemical compounds in a *Baptisia* hybrid population. *Nature* **184**, 285-286.